SHORT REPORT

Urinary *chiro*-Inositol Excretion is an Index Marker of Insulin Sensitivity in Japanese Type II Diabetes

SUSUMU SUZUKI, MD
HIROMASA KAWASAKI, MD
YOSHINORI SATOH, MD
MASATAKA OHTOMO, MD
MASASHI HIRAI, MD
AKI HIRAI, MD
SATOSHI HIRAI, MD
MASATOSHI ONODA, MD
MASAHIRO MATSUMOTO, MD
YOSHINORI HINOKIO, MD
HIROAKI AKAI, MD
JAMES CRAIG, MD
JOSEPH LARNER, MD, PHD
TAKAYOSHI TOYOTA, MD

OBJECTIVE — To determine the relationship between urinary *chiro*-inositol excretion and insulin sensitivity in Japanese type II diabetic patients.

RESEARCH DESIGN AND METHODS — Eighteen subjects were age-matched, nonobese, type II diabetic patients. Eight subjects had impaired glucose tolerance (IGT), and 10 had normal glucose tolerance (NGT). We quantified urinary *chiro*-inositol excretion using gas chromatography-mass spectrometry and the insulin sensitivity index (S_I), and glucose effectiveness (S_G) using Bergman's modified minimal model method.

RESULTS — The urinary excretion of *chiro*-inositol was much lower in the diabetic patients (32.3 ± 16.0 μmol/day, means ± SD) than in the NGT subjects (96.0 ± 17.6; $P < 0.0001$) and IGT subjects (58.9 ± 11.6; $P < 0.0001$). S_I was much lower in the diabetic patients (3.81 ± 1.49) than in the NGT subjects (6.30 ± 1.59, $P < 0.0005$). S_G was much lower in the diabetic patients (2.14 ± 0.56) than in the NGT subjects (3.07 ± 0.38, $P < 0.0001$). There was a significant correlation between urinary *chiro*-inositol excretion and S_I ($r = 0.766$), as well as a significant correlation between urinary *chiro*-inositol excretion and S_G ($r = 0.747$).

CONCLUSIONS — There is a direct relationship of urinary *chiro*-inositol excretion to insulin sensitivity and S_G in humans. Urinary *chiro*-inositol excretion might be useful as a metabolic index of insulin sensitivity in type II diabetes.

From the Third Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan; and the Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia.

Address correspondence and reprint requests to Susumu Suzuki, MD, Third Department of Internal Medicine, Tohoku University School of Medicine, 1–1 Seiryou-machi, Aoba-ku, 980–77 Sendai, Japan.

Received for publication 8 April 1994 and accepted for publication 30 June 1994.

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; S_I, insulin sensitivity index; S_G, glucose effectiveness; BMI, body mass index.

The molecular mechanism of insulin resistance in type II diabetes is not well known. Insulin binding to its receptor induces hydrolysis of phosphatidylinositol-glycan and releases inositol-glycan and diacylglycerol, two putative second messengers of insulin actions (1–3). *Chiro*- and *myo*-inositols are major components of the inositol-glycans and phosphatidylinositol-glycans (4–7). Kennington et al. (5) reported hypochiroinositoluria in type II diabetic subjects and decreased *chiro*-inositol in mediator prepared from skeletal muscle biopsies of Pima Indian diabetic subjects together with increased urinary *myo*-inositol excretion. Ortmeyer et al. (6) demonstrated a direct relationship of insulin resistance, as measured by five parameters of insulin action to urinary *chiro*-inositol excretion in the type II diabetic rhesus monkey (*Macaca mulatta*). Nonobese type II diabetic GK (Goto-Kakizaki) rats have been shown to manifest insulin resistance, decreased insulin action, and strikingly decreased urine *chiro*-inositol together with increased urine *myo*-inositol (7). We investigated the possible linkage of hypochiroinositoluria to insulin resistance in Japanese type II diabetic patients.

RESEARCH DESIGN AND METHODS

— The study protocol was approved by the Tohoku University Institutional Review Board. Informed consent was obtained from each subject. The subjects were age-matched, nonobese, subjects with normal glucose tolerance (NGT) ($n = 10$), subjects with impaired glucose tolerance (IGT) ($n = 8$), and type II diabetic patients ($n = 18$), as shown in Table 1. All subjects were free of hypertension, hyperlipidemia, and ischemic cardiovascular diseases and had normal 24-h creatinine clearance. All diabetic patients, IGT subjects, and NGT subjects fulfilled the World Health Organization criteria for diabetes. IGT and NGT sujects were evaluated within a year by 75 g oral glucose tolerance tests (8). All diabetic patients were classified as having type II

Table 1—*Clinical characteristics, S_I, S_G, and 24-h urinary excretion of* chiro-*inositol and* myo-*inositol in study subjects*

Subjects	NGT	IGT	Diabetes
n	10	8	18
Sex (M/F)	5/5	4/4	10/8
Age (years)	51.0 ± 17.0	52.2 ± 13.2	55.9 ± 10.2
BMI	21.1 ± 1.2	22.0 ± 1.2	22.4 ± 1.4
Plasma C-peptide after glucagon (ng/ml)	6.66 ± 2.66	5.61 ± 2.77	4.42 ± 1.60*
Urinary C-peptide excretion (ng/day)	102.6 ± 31.2	86.6 ± 32.6	70.4 ± 19.9†
S_I ($\times 10^{-4} \cdot min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$)	6.30 ± 1.59	4.80 ± 0.58‡	3.81 ± 1.49§
S_G ($\times 10^{-2}$ min)	3.07 ± 0.38	2.55 ± 0.50‖	2.14 ± 0.56¶
Urinary *chiro*-inositol excretion (μmol/day)	96.0 ± 17.6	58.9 ± 11.6#	32.3 ± 16.0[a,b]
Urinary *myo*-inositol excretion (μmol/day)	192 ± 54	212 ± 55	499 ± 112[c,d]

Data are means ± SD. * $P < 0.01$ (NGT vs. diabetes). † $P < 0.005$ (NGT vs. diabetes). ‡ $P < 0.05$ (NGT vs. IGT). § $P < 0.0005$ (NGT vs. diabetes). ‖ $P < 0.05$ (NGT vs. IGT). ¶ $P < 0.0001$ (NGT vs. DM). # $P < 0.0002$ (NGT vs. IGT). [a] $P < 0.0001$ (NGT vs. diabetes). [b] $P < 0.0005$ (IGT vs. diabetes. [c] $P < 0.0001$ (NGT vs. DM). [d] $P < 0.0001$ (IGT vs. diabetes).

diabetes because their plasma C-peptide level 6 min after a 1 mg glucagon injection was >2 ng/ml (4.42 ± 1.60 ng/ml), as well as their urinary C-peptide excretion for 24 h was >40 ng/day (70.4 ± 19.9 ng/day) as shown in Table 1. Diabetic patients were free of ketoacidosis episodes and diabetic complications except for minimal background retinopathies. Diabetic patients were treated with diet therapy ($n = 8$) or oral hypoglycemic agents ($n = 10$) and were under stable control, as means ± SD HbA_{1c} concentrations in the diabetic patients were 6.51 ± 0.62%.

Gas chromatography-mass spectrometry determination of 24-h *chiro*-inositol excretion

We collected urine samples for 24 h, which were frozen until processed for analysis. Aliquots (5 ml) were passed through 1-ml mixed bed ion-exchange resins (Amberlite MB-3) to remove ionic substances and then through a C_{18} Sep-Pak cartridges (Waters) to remove nitrogenous, hydrophobic, or peptide-like materials. The inositols and other neutral hydrophilic materials were eluted from the Sep-Pak cartridges with 2 ml water and lyophilized. The lyophilized samples were derivatized with 100 μl of heptafluorobutyrylimidazole (Pierce) and heated at 55°C for 5 h. The samples were then extracted into 200 μl *n*-hexane and subjected to gas chromatography-mass spectrometry analysis, as described by Kennington et al. (5). All samples were analyzed twice, and the results reported are the average of the two analyses (rate of error <6%).

Minimal model study

The insulin sensitivity index (S_I) and glucose effectiveness (S_G) were assessed by the use of the modified Bergman's minimal model method with an additional administration of insulin 20 min after the glucose bolus (0.3 g/kg), as described by Welch et al. (9). The NGT, IGT, and diabetic subjects received 0.02 U/kg regular human insulin. Plasma glucose was assayed using the glucose oxidase method. Plasma insulin and C-peptide were assayed using radioimmunoassay kits.

Statistical analysis

Data are expressed as means ± SD. Statistical analysis was made by means of the unpaired Student's *t* test. $P < 0.05$ was considered statistically significant.

RESULTS— The urinary excretion of *chiro*-inositol was much lower in the type II diabetic patients (32.3 ± 16.0 μmol/day) than in the NGT (96.0 ± 17.6; $P < 0.0001$) and IGT subjects (58.9 ± 11.6; $P < 0.0005$), as shown in Table 1. The urinary *chiro*-inositol excretion was much lower in the IGT subjects than in the NGT subjects ($P < 0.0002$). The mean urinary *myo*-inositol excretion was higher in the diabetic patients (499 ± 112 μmol/day; $P < 0.0001$) than in the IGT subjects (212 ± 55) and NGT subjects (192 ± 54) (Table 1). S_I in the NGT, IGT, and diabetic subjects were 6.30 ± 1.59, 4.80 ± 0.58, and 3.81 ± 1.49 $\times 10^{-4}$ $min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$, respectively (Table 1). S_I was much lower in the diabetic patients than in the NGT subjects ($P < 0.0005$). S_I was also lower in the IGT subjects than in the NGT subjects ($P < 0.05$). There was a significant correlation between urinary *chiro*-inositol excretion and S_I ($r = 0.766$), as shown in Fig. 1. S_G in the NGT, IGT, and diabetic subjects were 3.07 ± 0.38, 2.55 ± 0.50, and 2.14 ± 0.56 ×

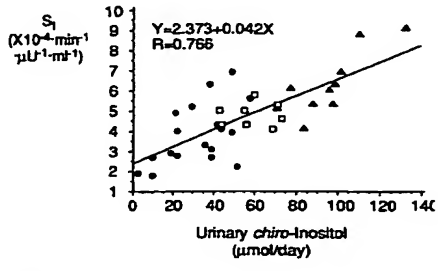

Figure 1—*Relationship between urinary excretion of* chiro-*inositol for 24 h and S_I. ●, type II diabetic patients; □, IGT subjects; ▲, NGT subjects.*

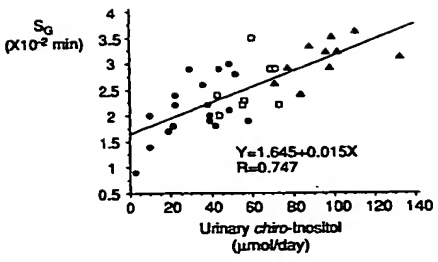

Figure 2—*Relationship between urinary excretion of* chiro-*inositol for 24 h and S_I. ●, type II diabetic patients; □, IGT subjects; ▲, NGT subjects.*

10^{-2} min, respectively (Table 1). S_G was lower in the diabetic patients than in the NGT subjects ($P < 0.0001$). S_G was also lower in the IGT subjects than in the NGT subjects ($P < 0.05$). There was also a significant correlation between urinary *chiro*-inositol excretion and S_G ($r = 0.747$), as shown in Fig. 2. There was no correlation between urinary *chiro*-inositol excretion and the age, sex, and body mass index (BMI) of three groups (data not shown). There was a weak correlation between urinary *chiro*-inositol excretion and plasma C-peptide concentrations 6 min after glucagon injection ($r = 0.581$) (data not shown). There was a significant correlation between plasma C-peptide concentrations 6 min after glucagon injection and 24-h urinary C-peptide excretion ($r = 0.681$); however, there were no correlation between urinary *chiro*-inositol excretion and 24-h urinary C-peptide excretion (data not shown). There was no significant correlation between urinary *chiro*-inositol and *myo*-inositol excretion (data not shown).

CONCLUSIONS— Peripheral insulin resistance of glucose metabolism in type II diabetes has been characterized as postreceptor, predominantly in nonoxidative glucose metabolism, directly correlated with a decreased ability of in vivo insulin to activate muscle glycogen synthase, and yielding a decreased glucose metabolism rate (10,11). The fact that decreased muscle glycogen synthase activation has been observed in first-degree relatives who are not diabetic suggests a familial or possibly genetic basis for the resistance (10,11). Bergman's modified minimal model method using exogenous insulin has been applied to estimate simultaneously S_I and S_G in normal subjects and type II diabetic patients (9,12–14). Values of S_I obtained by the modified minimal model method were highly correlated with the S_I measured using the euglycemic glucose clamp method (12). S_G, the ability of glucose per se, independent of changes in insulin, to increase glucose uptake and suppress endogenous output, is commonly reduced in type II diabetes (12). The defects in S_I and S_G are synergistic in causing glucose intolerance in type II diabetic and IGT subjects (12).

This study demonstrates a significant correlation between urinary *chiro*-inositol excretion and S_I, as well as S_G, and a weak correlation between urinary *chiro*-inositol excretion and plasma C-peptide concentrations 6 min after glucagon injection, but no significant correlation between urinary *chiro*-inositol excretion and 24-h urinary C-peptide excretion. These data suggest a causal relationship between urinary *chiro*-inositol excretion and in vivo insulin resistance. A significant correlation between urinary *chiro*-inositol excretion and in vivo insulin resistance was reported in type II diabetic rhesus monkeys (5). Hypochiroinositoluria and insulin resistance were also demonstrated in GK rats (7). Asplin et al. (15) documented decreased inositol-glycan bioactivity and *chiro*-inositol content in the inositol-glycan fractions isolated from hemodialysate, urine, and autopsy muscle of type II diabetic subjects. In contrast, the *myo*-inositol-containing inositol-glycan activities from the three sources were indistinguishable when nondiabetic and type II diabetic subjects were compared (12). Ortmeyer et al. (16) demonstrated acute hypoglycemic effects of oral D-*chiro*-inositol administration in streptozotocin-induced diabetic rats and diabetic rhesus monkeys (16). We recently found that oral administration of D-*chiro*-inositol for a month normalized the deficiency of *chiro*-inositol in skeletal muscle and liver of the diabetic GK rats and improved glucose intolerance in the rats, insulin sensitivity on glycogen synthesis, lipogenesis, and activation of pyruvate dehydrogenase in rat-isolated adipocytes (17). These studies provide evidence that *chiro*-inositol deficiency may be linked to peripheral insulin resistance in type II diabetes. Pak et al. (19) have demonstrated a pathway of *chiro*-inositol biosynthesis from *myo*-inositol in rats (18) and rat fibroblasts, which are stimulated by insulin. Nothing is known about the formation of *chiro*-inositol in humans. Defects of *chiro*-inositol biosynthesis or metabolism might contribute to insulin resistance, a key important early manifestation of type II diabetes. Low urinary excretion of *chiro*-inositol might be useful for evaluation of insulin resistance and impaired glucose utilization in type II diabetes.

Acknowledgments— This investigation was supported in part by a grant for diabetes research from the Ministry of Health and Welfare.

References

1. Saltiel AR, Fox JA, Sherline P, Cuatrecasas P: Insulin-stimulated hydrolysis of a novel glycolipid generates modulators of cAMP phosphodiesterase. *Science* 233: 967–972, 1986
2. Larner J: Lesson from the old testament of glycogen metabolism and the new testament of molecular biology. *Diabetes* 37: 262–275, 1988
3. Saltiel AR: The role of glycosyl-phosphoinositides in hormone action. *J Bioenerg Biomembr* 23:29–41, 1991
4. Larner J, Huang LC, Schwartz CFW, Oswald AS, Shen T-Y, Zeller K: Rat liver insulin mediator which stimulates pyruvate dehydrogenase phosphatase contains galactosamine and D-*chiro*-inositol. *Biochem Biophys Res Commun* 151:1416–1426, 1988

5. Kennington AS, Hill CR, Craig J, Bogardus C, Raz I, Ortmeyer HK, Hansen BC, Romero G, Larner J: Low urinary *chiro*-inositol excretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 323: 373–378, 1990
6. Ortmeyer HK, Bodkin NL, Lilly L, Larner J, Hansen BC: *Chiro*-inositol deficiency and insulin resistance. I. Urinary excretion rate of *chiro*-inositol is directly associated with insulin resistance in spontaneously insulin-resistant Rhesus monkey. *Endocrinology* 132:640–645, 1993
7. Suzuki S, Taneda Y, Hirai S, Abe S, Sasaki A, Suzuki K, Toyota T: Molecular mechanism of insulin resistance in spontaneous diabetic GK (Goto-Kakizaki) rats. In *New Directions in Research and Clinical Works for Obesity and Diabetes Mellitus*. Sakamoto N, Angel A, Hotta N, Eds. Amsterdam, Elsevier, 1991, p. 197–203
8. American Diabetes Association: Official guide to diagnosis and classification of diabetes mellitus and other categories of glucose intolerance. *Diabetes Care* 14: 3–4, 1991
9. Welch S, Gebhart S, Bergman R, Phillips L: Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. *J Clin Endocrinol & Metab* 71:1508–1518, 1990
10. DeFronzo R, Bonadonna R, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
11. Beck-Nielsen H, Vaag A, Damsbo P, Handberg A, Nielsen OH, Henriksen JE, Thye-Ronn P: Insulin resistance in skeletal muscles in patients with NIDDM. *Diabetes Care* 15:418–429, 1992
12. Bergman RN: Lilly Lecture 1989: toward physiological understanding of glucose tolerance: minimal-model approach. *Diabetes* 38:1512–1527, 1989
13. Taniguchi A, Nakai Y, Fukushima M: Pathogenic factors responsible for glucose intolerance in patients with NIDDM. *Diabetes* 41:1540–1546, 1992
14. Wajchenberg BL, Santomauro AT, Porrelli RN: Effect of a sulfonylurea (gliclazide) treatment on insulin sensitivity and glucose-mediated glucose disposal in patients with non-insulin-dependent diabetes mellitus (NIDDM). *Diabetes Res Clin Pract* 20:147–154, 1993
15. Asplin I, Galasko G, Larner J: *Chiro*-inositol deficiency and insulin resistance: a comparison of the *chiro*-inositol- and the *myo*-inositol-containing insulin mediators isolated from urine, hemodialysate, and muscle of the control and type II diabetic subjects. *Proc Natl Acad Sci USA* 90: 5924–5928, 1993
16. Ortmeyer HK, Huang LC, Zhang L, Hansen BC, Larner J: *Chiro*-inositol deficiency and insulin resistance. II. Acute effects of *chiro*-inositol administration in streptozotocin-diabetic rats, normal rats given a glucose load, and spontaneously insulin-resistant Rhesus monkey. *Endocrinology* 132:646–651, 1993
17. Suzuki S, Hirai S, Onoda M, Hinokio Y, Matsumoto M, Satoh Y, Akai H, Larner J, Toyota T: The effects of *chiro*-inositol replacement in spontaneously diabetic GK rats. *Diabetes Res Clin Pract.* In press
18. Pak Y, Huang LC, Lilley KJ, Larner J: In vivo conversion of [^{3}H]*myo*-inositol to [^{3}H]*chiro*-inositol in rat tissues. *J Biol Chem* 267:16904-16910, 1992
19. Pak Y, Paule CR, Bao YD, Huang LC, Larner J: Insulin stimulates the biosynthesis of *chiro*-inositol-containing phospholipids in rat fibroblast line expressing the human insulin receptor. *Proc Natl Acad Sci USA* 90:7759–7763, 1993